

Supplemental Figure Legends

Figure S1. Drug enrichment of silent mutations that did not change the parental BRAFV600E protein sequence. Enrichment of individual silent mutations was not reproducible and no silent mutations were enriched using a net false discovery threshold of 1% ($q=0.1$ in each replicate; $q1*q2 = 0.01$).

Figure S2. PLX4720 resistance of A375 cell lines expressing BRAFV600E, BRAFV600E/L505H, BRAFV600E/F516G or BRAFV600E/T529N. Cellular proliferation assay monitoring growth of A375 cells expressing BRAFV600E, BRAFV600E/L505H, BRAFV600E/F516G or BRAFV600E/T529N and treated with either DMSO (left) or 4 μ M PLX4720 (right). The results show that in the absence of PLX4720, proliferation of A375 cells expressing BRAFV600E was comparable to that of cells expressing BRAFV600E/L505H, BRAFV600E/F516G or BRAFV600E/T529N. Furthermore, A375 cells expressing BRAFV600E/L505H, BRAFV600E/F516G or BRAFV600E/T529N were all more resistant to PLX4720 compared to cells expressing BRAFV600E. Error bars indicate SD.

Figure S3. Phospho-MEK or phospho-ERK1/2 IC₅₀ curves for the immunoblots shown in Fig. 2B–E. (A–C) Phospho-MEK IC₅₀ of A375 cells stably transduced with BRAFV600E, BRAFV600E/L505H, BRAFV600E/F516G or BRAFV600E/T529N and treated with increasing doses of PLX4720 (A), SB5908885 (B) or RAF265 (C). (D) Phospho-ERK1/2 IC₅₀ of A375 cells stably transduced with BRAFV600E, BRAFV600E/L505H, BRAFV600E/F516G or BRAFV600E/T529N and treated with increasing doses of U0126. The phospho-MEK and phospho-ERK1/2 immunoblots were quantified, and the densitometry used to plot the data.

Figure S4. Analysis of ERK target gene expression following drug treatment in A375 cell lines expressing BRAFV600E, BRAFV600E/L505H, BRAFV600E/F516G or BRAFV600E/T529N. (A–C) qRT-PCR analysis monitoring expression of ERK target genes *FOSL1* (A), *SPRY2* (B) and *DUSP6* (C) in A375 cells stably transduced with BRAFV600E, BRAFV600E/L505H, BRAFV600E/F516G or BRAFV600E/T529N and treated with 20 μ M PLX4720, 0.8 μ M SB5908885, 2.4 μ M RAF265 or 4 μ M U0126. Error bars indicate SD.

Figure S5. Relative drug resistance of BRAFV600E mutants in A375 cells, and confirmation of PLX4720 resistance of the BRAFV600E/L505H mutant in an additional BRAFV600E-positive human melanoma cell line. (A–D) Cellular proliferation assays of A375 cells stably transduced with BRAFV600E, BRAFV600E/L505H, BRAFV600E/F516G or BRAFV600E/T529N and treated with PLX4720 (A), SB5908885 (B), RAF265 (C) or U0126 (D). (E) Cellular proliferation assay of MALME-3M cells stably transduced with BRAFV600E or BRAFV600E/L505H and treated with PLX4720. Error bars indicate SD.

Figure S6. Increased PLX4720 resistance of A375 cells expressing BRAFV600E. Cellular proliferation of parental A375 cells (–) or A375 cells stably transduced with BRAFV600E and treated with increasing doses of PLX4720. The results show that A375 cells transduced with BRAFV600E were approximately 6-fold more resistant to PLX4720 compared to parental A375 cells.

Figure S7. Phospho-MEK or -ERK1/2 IC₅₀ curves for the immunoblots shown in Fig. 3B, C. (A) Phospho-MEK IC₅₀ of 293T cells expressing BRAFV600E or BRAFV600E/L505H and treated with increasing doses of PLX4720. (B) Phospho-ERK-1/2 IC₅₀ of 293T cells expressing BRAFV600E or BRAFV600E/L505H and treated with increasing doses of U0126. The phospho-

MEK or phospho-ERK1/2 immunoblots were quantified, and the densitometry used to plot the data.

Figure S8. Phospho-MEK IC₅₀ curve for the immunoblots shown in Fig. 4C. Phospho-MEK IC₅₀ of Ba/F3 cells stably expressing BRAFV600E or BRAFV600E/L505H and treated with increasing doses of PLX4720. The phospho-MEK immunoblots were quantified, and the densitometry used to plot the data.

Figure S9. Confirmation of the identity of the Ba/F3 cell line used in this study. (A) IL-3 withdrawal experiment. 0.1×10^6 Ba/F3 cells were seeded in 6-well plates and cultured in the absence or presence of IL-3 (at a final concentration of 5 ng/ml). Cell number was monitored by Trypan Blue assay. Error bars indicate SD. (B) FACS analysis. Ba/F3 cells, and in parallel, as a control, murine IL-3 dependent myeloid 32D cells, were stained with CD11b and F4/80 and analyzed by FACS. Consistent with previously reported results (see http://www.brc.riken.jp/lab/cell/english/rcb0805_announce.shtml), we find that our Ba/F3 cells stained positively for F4/80 but not CD11b, whereas 32D cells stained positively for both F4/80 and CD11b. Collectively, these results confirm the identity of the Ba/F3 cell line used in this study.